

Structure-function Relationships  
in the Marine Diatom, Striatella  
unipunctata.

by

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Con mis mas profundos agradecimientos y con todo  
mi cariño, dedico esta tesis a mi vieja, Eva.

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## CHAPTER I.

Cytoplasmic Fine Structure With  
Emphasis on the Golgi Apparatus.

## INTRODUCTION

Striatella unipunctata, a marine pennate diatom, belongs to the Division Chrysophycophyta (3) which includes algae with brown pigment, a cell wall composed of silica with two distinct halves joined by loop-like structures. Striatella belongs to the class Bacillariophyceae, which comprises organisms with bilateral symmetry in both girdle and valvar views. Striatella is non-motile and therefore has pseudoraphes. Mucilagenous material formed at one apex of the cell serves as a holdfast for organisms in the subgroup Araphidineae which includes Striatella (17).

Studies on Striatella unipunctata performed in the last one hundred years are unknown to the author. Fritsch (11) cites references only of a general diatom nature in the 1880's and 1890's in regard to this organism. This study was originally undertaken to characterize the organism in preparation for further work. However the findings also allow particular emphasis to chloroplast movement, microtubule-nuclear envelope relationship and Golgi pairing related to nuclear blebbing.

## MATERIALS AND METHODS

Striatella unipunctata was isolated by Dr. L. E. Roth from the shallow low-tide waters off Woods Hole, Massachusetts in August, 1974. A second collection was also successfully made in the spring of 1975. Cultures were maintained in petri dishes with 80% Instant Ocean synthetic sea water at a salinity of 30 parts/million under a 14-10 hour, light-dark cycle and at temperatures ranging from 14° C to 22° C. Culturing techniques were perfected to the point where cloning of individual organisms was possible. For this repeated transfer of individual cells through a series of small petri dishes was performed with a micropipette to assure that one single individual was in each dish without contaminating flagellates or ciliates. Fresh medium was added twice a week, after decanting the old medium, and rinsing the dish twice.

Cultures were usually chosen for fixation at the beginning of the dark period with the hope of obtaining a comparatively high number of organisms in or near division. Light-dark synchrony has been studied and successfully utilized by other investigators (14, 19, 20) and seemed to be the technique of choice for this study since it allowed division synchrony in the organisms under the most natural conditions. Synchrony in a silica-free medium (20) was tried unsuccessfully; for this, the cells were grown in plastic petri dishes with Instant Ocean synthetic silica-free sea water, but the levels of synchrony were very low, and cultures appeared to be adversely affected very quickly.

After the organisms were partially synchronized with the light-dark technique and their division stages determined by light microscopy, clean clones were fixed in the petri dishes where they were cultured and to which

they had firmly and naturally attached. As the investigation proceeded, minor modifications of the preservation schedule were made.

After the culture fluid had been decanted, 2% glutaraldehyde prepared in 75% synthetic sea water was added for 5-20 minutes at room temperature. This solution was decanted, and the organisms were postfixed in 1%  $\text{OsO}_4$ , in 75% synthetic sea water for 25-35 minutes at room temperature. In some of the fixation runs,  $2 \times 10^{-3}$  M sucrose was added to the glutaraldehyde and osmium solutions but had little if any influence on the quality of fixation. Another modification was the use of an "Osmette A" osmometer to adjust the osmolarity of the glutaraldehyde and Osmium solutions to the osmolarity of the growth medium which was 834 milliosmols. This procedure was employed for the last fixation run performed, but no improvement over the previous fixations was observed.

After the postfixation, the cells were slowly dehydrated through 50%, 50%, 75%, 95%, and 100% acetonitrile in 5-7 minute steps. Subsequently the cells were placed in 3:1 100% acetonitrile:Spurr's low-viscosity resin for 15-80 minutes. The percentage of the Spurr's resin was increased to 50% after 40-120 minutes and to 75% after 20-120 more minutes, until the material was in 100% Spurr for 20-120 minutes. The organisms were then transferred to fresh Spurr's medium for 1-2 hours before embedding in BEEM capsules or flat embedding molds. Polymerization was done at 60° C overnight (15-18 hrs.).

The fixed cells were clearly visible through the resin, and the flat embeddings were examined under a Zeiss R. A. microscope by using standard bright-field techniques. Individual cells were selected according to their division stage, and their location was designated with a diamond marker attachment that scratched a ring on the resin around the cell. These individuals were dissected from flat embedding with a razor blade and glued with

Epoxy cement on a stub. This procedure allowed positioning of individual cells exactly in chosen positions so that known section orientations were possible; usually the valvar plane (Fig. 1b) or the girdle plane (in the plane of the page of Fig. 2) were chosen to study the fine structure of Striatella at cell division.

The BEEM capsule embeddings were also trimmed to examine many organisms in the same section without known orientation.

Gold to silver sections were cut on a Reichert OmU-2 ultramicrotome with a Dupont diamond knife, picked up on formvar-covered 200-mesh copper grids, stained with 5% uranyl acetate in 50% ethanol followed by lead citrate for 15 and 7 minutes respectively and examined in a Phillips EM 201 electron microscope operated at 60 Kv. Electron micrographs were taken with Kodak ESTAR-4489 8.3 x 10.2 cm. film.



## RESULTS

Striatella unipunctata forms loosely packed groups of organisms randomly distributed throughout and attached to the culture dish. The organisms secrete a mucilaginous substance which both forms a holdfast by which they adhere firmly to the substratum and causes organisms to stick together.

Individual cells are enclosed within a siliceous frustule which is composed of two valves, numerous intercalary bands, and girdle bands and which, by light microscopy looks like a translucent rectangular pillow (Fig. 1a, 1b, 2). A detailed description of the frustule is presented in chapter two of this study.

By the time of cell division, a clear belt, the girdle, is located in the central region of the frustule (Fig. 2), and the cell divides at the girdle along a plane parallel to the valves. After mitosis new valves are secreted by the daughter cells on the surface of the freshly formed protoplasm.

### Polar plates and stalks.

Four polar plates, one in each corner of the cell, are located on opposite ends of both valves (Fig. 1a). Up to these plates, cytoplasm is seen in some cases (Fig. 3). In other cases when the holdfast is being secreted, the cytoplasm does not extend to the plate. Rather, material seen first in intracellular vesicles (Fig. 4, Mv) and later in extracellular areas (Fig. 4) seems to be extruded through the pores of the polar plate to form the stalk. Of the four polar plates, only one is used for the secretion of a stalk by a given organism. The fibrous nature of the stalk is apparent in Fig. 5.



Fig. 1a. Schematic diagram of the siliceous frustule of S. unipunctata, showing the organism at the time of cell division. The epitheca (E) and hypotheca (H) subdivided by intercalary bands (IB), are separated by the girdle (GR). Two valves (VL) contain the polar plates (PP) and are divided by a pseudoraphe (PR).

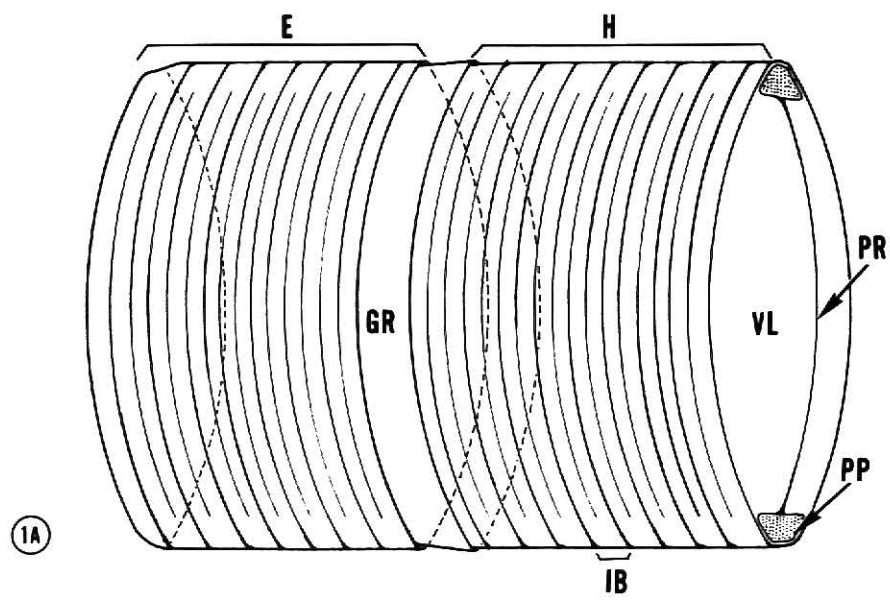




Fig. 1b. Schematic diagram of a section in valvar view. The nucleus (N) with its nucleolus (NO), nuclear envelope (NE), and annuli (NA) is centrally located. Golgi bodies (G) surround the nucleus. Chloroplasts (C) and mitochondria (M) are mainly located in the central region. Large vacuoles (V) separate the central cytoplasm (CT) from the siliceous frustule (F). The plasma membrane (PM) is found underneath the frustule.

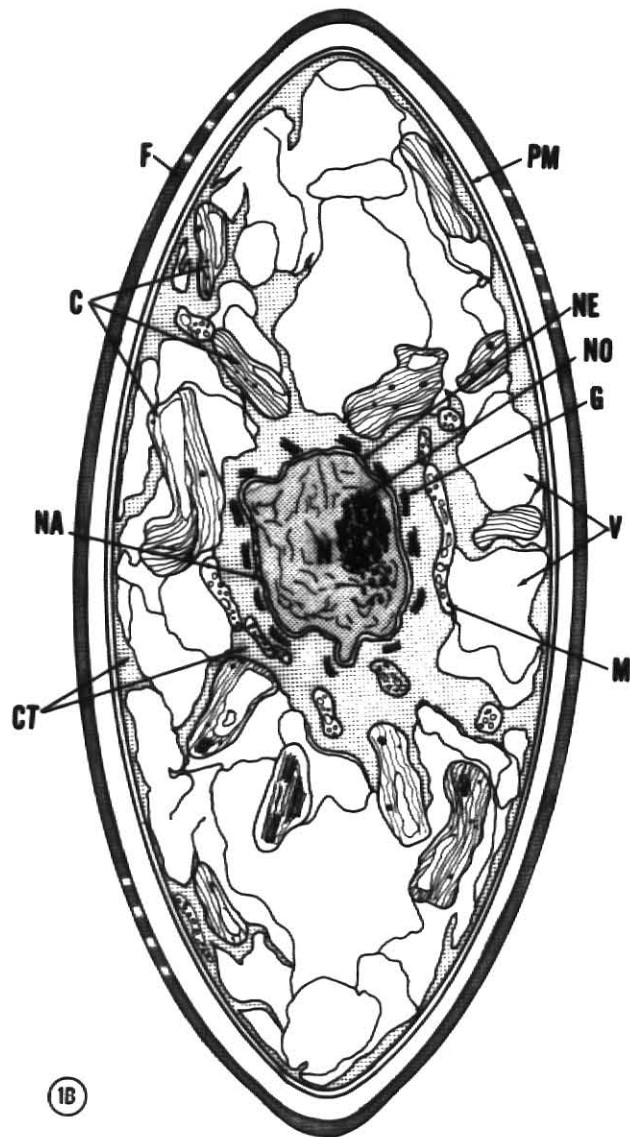






Fig. 2. Light micrograph of S. unipunctata shows the siliceous frustule to be composed of a girdle (GB) and numerous intercalary bands (IB). The valves (VL) run parallel to the girdle. A centrally located nucleus (N) is surrounded by chloroplasts (C). X 850.

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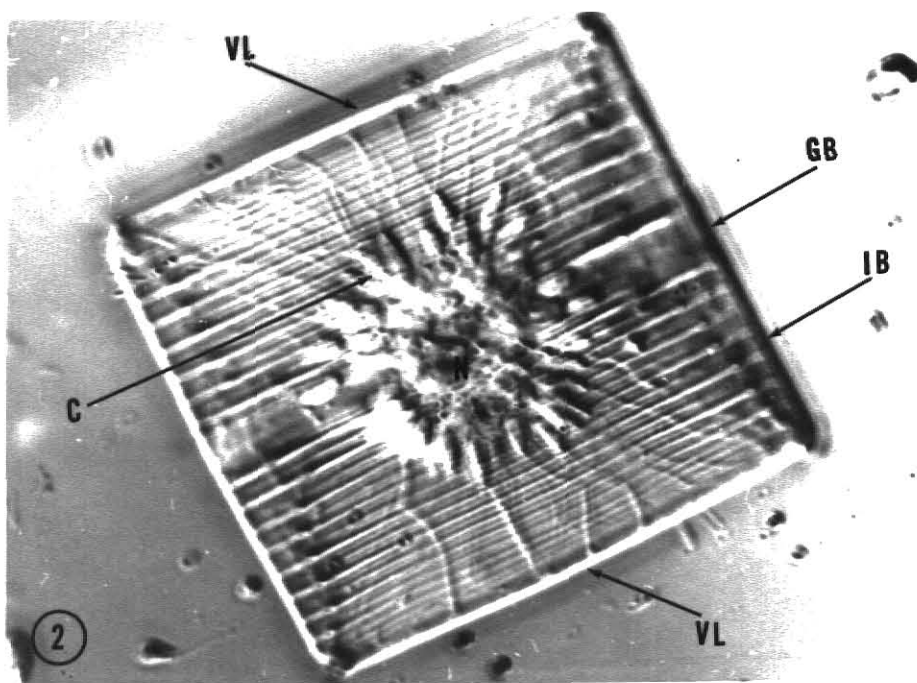
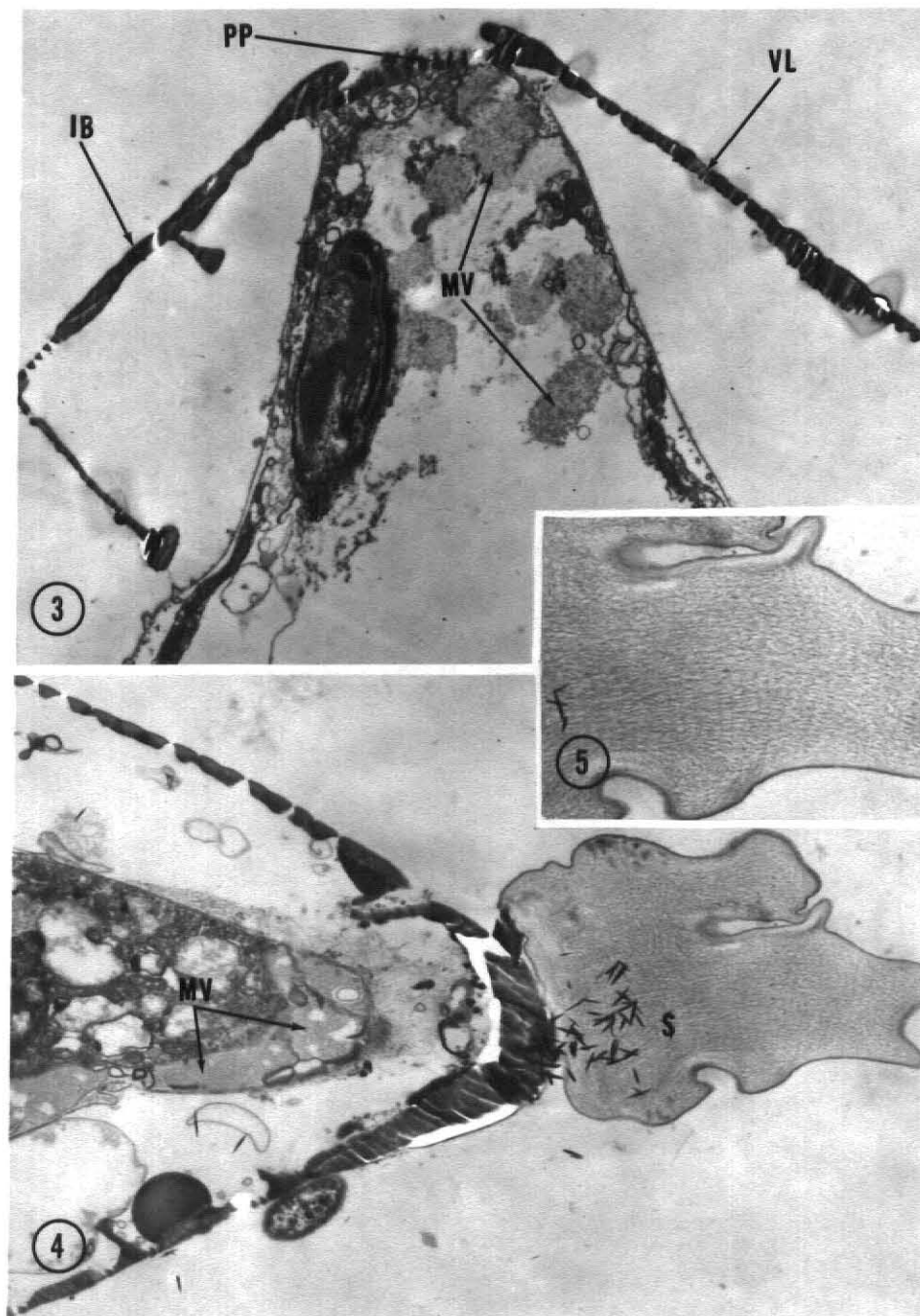




Fig. 3. Section through a polar plate of S. unipunctata shows portions of intercalary bands (IB) on one side and a valve on the other. The cytoplasm containing mucilagenous vesicles (MV) extends up to the polar plate (PP) where large pores can be seen. X 10,000.

Fig. 4. Section through a polar plate showing the secretion of the mucilagenous vesicles (MV) that forms the stalk (S). X 13,000.

Fig. 5. Detail of the stalk showing its fibrous nature. X 20,000.



### Chloroplasts.

In the cytoplasm, numerous yellow-brown chloroplasts occur peripherally near the vacuoles and beneath the siliceous frustule (Fig. 1b, 6-8). They also are seen radially oriented toward the central cytoplasm (Fig. 2). The distance between the chloroplasts and the central cytoplasm varies with the light intensity and quality. Bright white light causes the chloroplast to aggregate in the central cytoplasm near the nucleus; the phenomenon has therefore been termed karystrophy (Fig. 2). A time course for this reaction has been repeatedly observed to be about seven minutes. Observation of organisms in bright red light can be carried on for extended periods of time without initiating the reaction, and the reversal to a dispersed distribution can be accomplished by using lesser illumination if red light is liberally contained, though the time course is much longer.

A careful search for organelles that might generate such movement revealed fibrous structures interposed between the chloroplast and the cytoplasmic membrane. These organelles are probably microfilament bundles (Fig. 7, 8). A microtubule can be seen rarely in adjacent cytoplasmic strands (Fig. 8, MT). Preliminary experiments show this chloroplast movement to be inhibited by cytochalasin B but not by colchicine (16), results that suggest microfilament-related, not microtubule-related, motile forces.

Electron micrographs of the chloroplasts reveal very thin strands of cytoplasm surrounding but not enclosing them completely (Fig. 6, 7). These thin strands vary in size. On occasion, mitochondria, small vesicles, and ribosomes are buried in the cytoplasmic strands (Fig. 6-8); in other instances, the cytoplasmic strands consist simply of two closely opposed membranes (Fig. 6, 7).

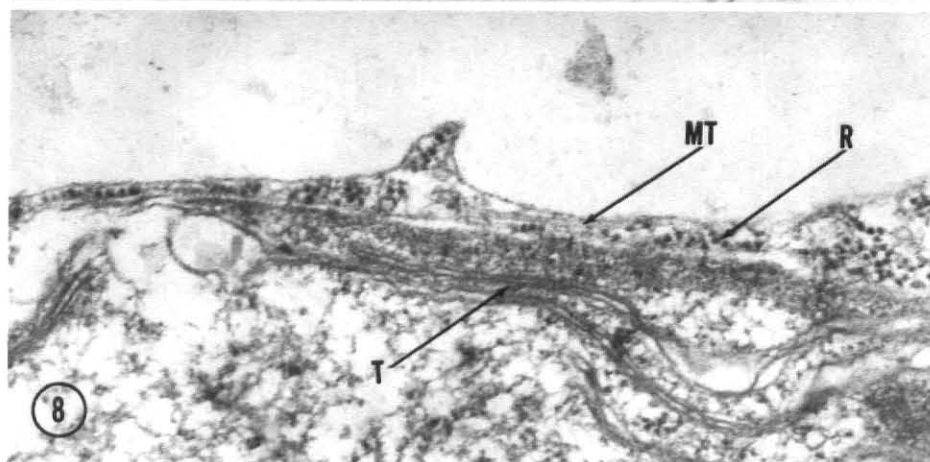
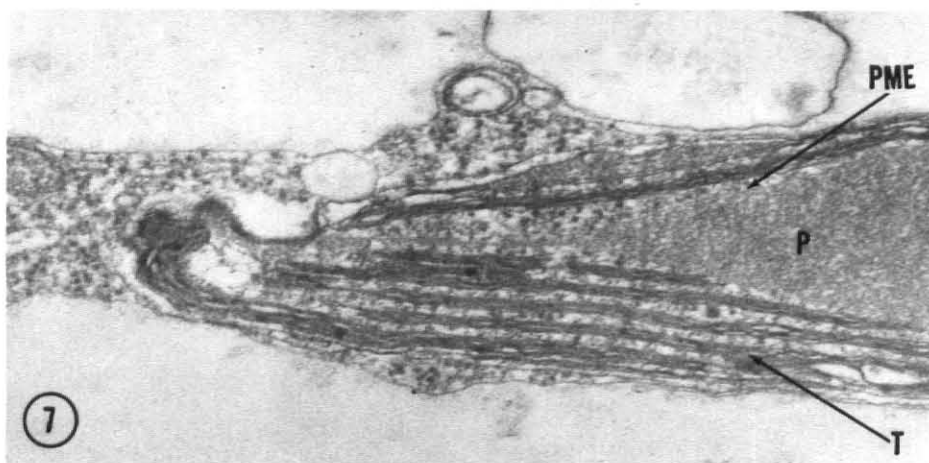
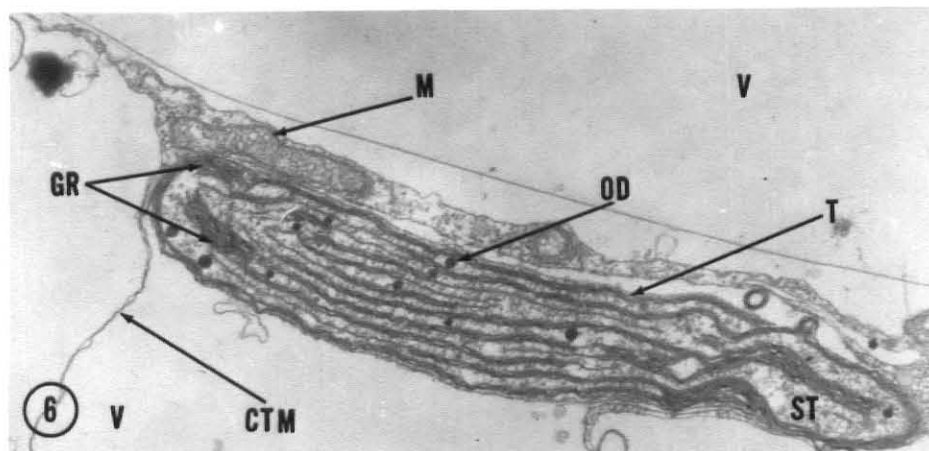




Fig. 6. Survey picture of a chloroplast of Striatella. A cytoplasmic sheet has projections of its thin cytoplasmic membrane (CTM), and encloses large vacuoles (V). The chloroplast contains stroma (ST), thylakoids (T), oil droplets (OD), and grana (GR). A mitochondrion (M) is found in the cytoplasmic strand. X 22,000.

Fig. 7. A chloroplast of S. unipunctata with a large pyrenoid (P) and its pyrenoid membrane (PME). The thylakoids (T) run on either side of the pyrenoid. Immediately above the chloroplast, a microfilament bundle is seen. X 57,000.

Fig. 8. A chloroplast with its typical thylakoids (T), surrounded by cytoplasm containing ribosomes (R) and a microtubule (MT). A microfilament bundle is positioned between the chloroplast and the cytoplasmic strand. X 57,000.



The chloroplasts themselves appear in sections as ellipsoidal bodies, membrane-bounded with very few grana (Fig. 6). The thylakoids or lamellae are clearly seen as continuous membranes enclosing an interior space and usually occur in stacks of only two, often as closely fused as in a typical granum. Some thylakoids occur singly (Fig. 6). The thylakoid space appears very irregular (Fig. 6-8). The stroma or matrix in which the membranes are suspended, is loaded with electron-dense particles (Fig. 6-8), some of which may be ribosomes (Fig. 8, R). Darkly stained oil droplets are also found in the stroma (Fig. 6). If the plane of section produces good thylakoid cross sections in one chloroplast, the others are similarly oriented. Since most of the culture conditions consisted of light vertically illuminating diatoms lying flat on the dish, it is likely the thylakoids are generally oriented parallel to the incident light. Pyrenoids are present and defined by a membrane (Fig. 7).

#### Microtubules and nuclear envelope.

Microtubules are common near to or outside of the nuclear envelope in cells chosen to be in or near karyokinesis (Fig. 9-11). None can be seen inside the nucleus at any time. On rare occasions, microtubules are seen near chloroplasts (Fig. 8). In some instances, microtubules occur in small groups close together and parallel to each other (Fig. 9, 11), but generally they do not show a precisely defined orientation; rather they appear to form a thin felt-work layer over the nucleus. Thus microtubules were chiefly found in the mitotic apparatus which is extranuclear, so that microtubules are excluded by the nuclear envelope from direct interaction with chromosomes. Some sheaves of microtubules are associated with nuclear deformation since they seem to push into the nucleus and form furrow-like indentations (Fig. 9, 10, 12, 13).



Fig. 9. Nuclear annuli (NP) in cross section, associated with microtubules (MT) parallel to the envelope. X 23,000.

Fig. 10. Oblique section of the nuclear envelope showing nuclear pores or annuli (NP). Microtubules (MT) oriented in a thin layer are found outside the nucleus. X 52,000.

Fig. 11. Microtubules (MT) running parallel to each other border the cross sectioned nuclear envelope with its pores (NP). X 73,000.

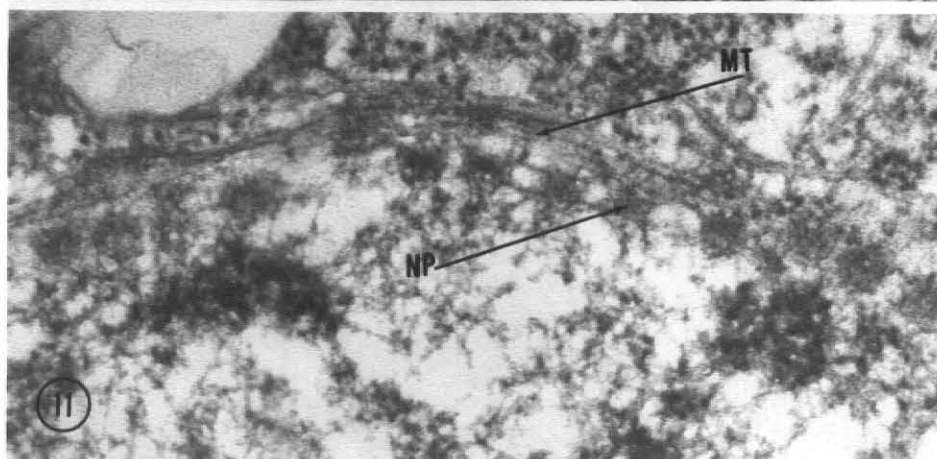
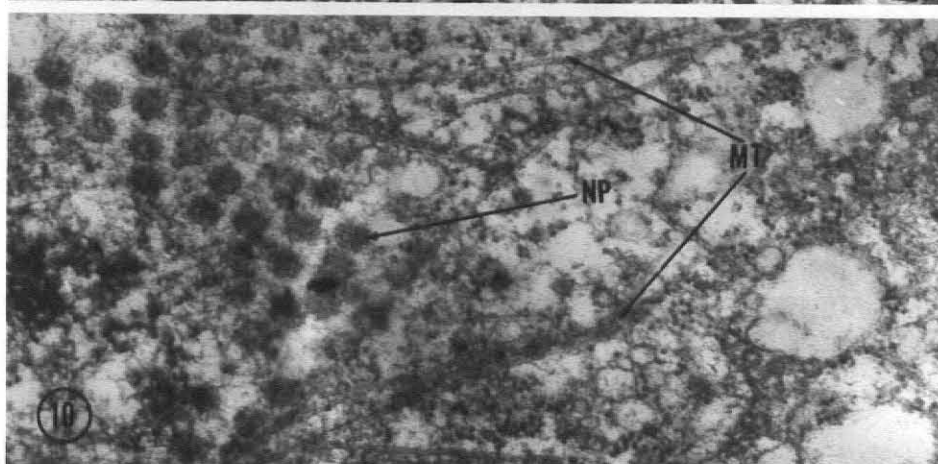
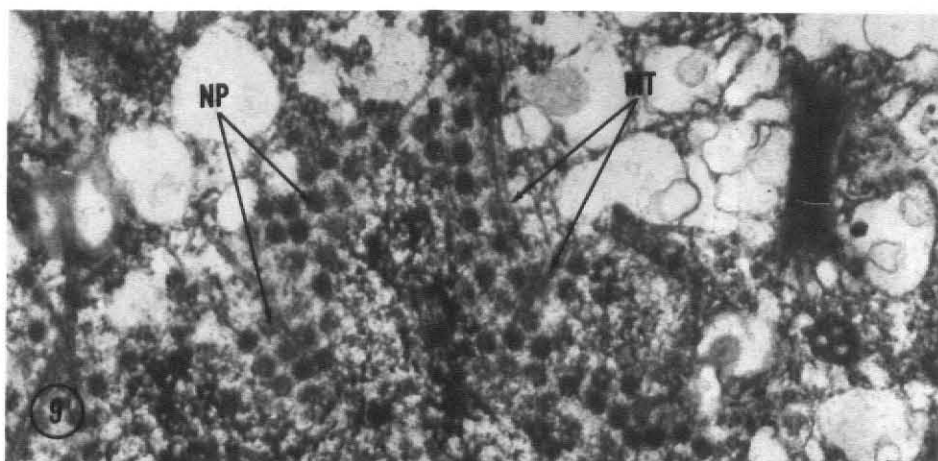
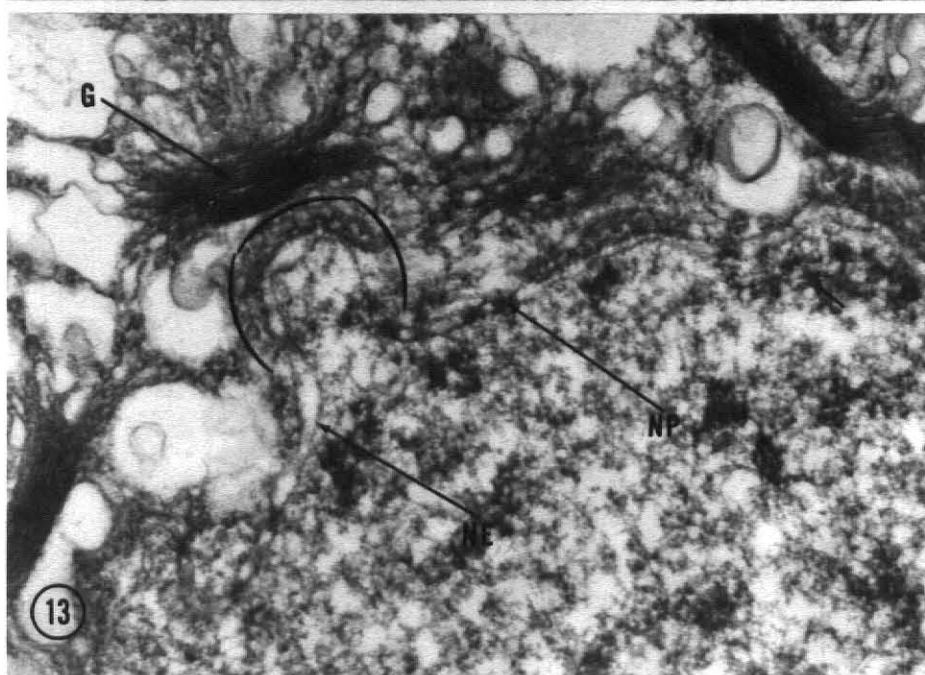
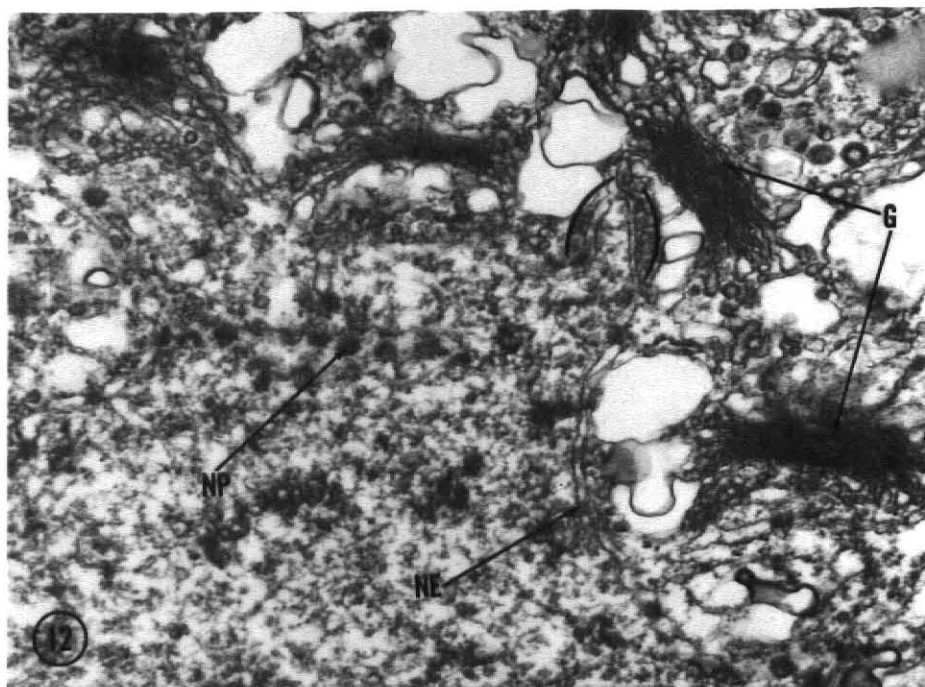




Fig. 12. Cross section through the nucleus. A folding of the nuclear envelope (NE) is indicated near a dictyosome (see area enclosed by curved lines). Some cross sections of the nuclear pores (NP) can be seen. Golgi bodies (G) surround the nucleus. X 26,000.

Fig. 13. Cross section of the convoluted nuclear envelope (NE) (see enclosed area) with nuclear pores (NP). A Golgi body (G) associates with the evaginations of the nuclear envelope. X 42,000.





The nuclear envelope has a typical double-membrane structure which contains comparatively high numbers of pores and annuli in all nuclei observed. Annuli were not observed in Striatella in the clear sense of showing discrete rings in cross section; rather the structures here observed appear to be more solid than most annuli. The nuclear membrane appears to remain intact throughout the cell cycle. A nuclear-envelope association with Golgi bodies is usually close and frequent to the extent that blebbing of the nuclear envelope results in the formation of vesicles directly related to the development of Golgi bodies (Fig. 14-16).

#### Golgi bodies.

The Golgi bodies or dictyosomes (Fig. 12-18) are found mainly in the central cytoplasm, grouped in such high numbers around the nucleus that a dictyosome zone is obvious, a characteristic of all diatoms studied (7). Individual dictyosomes are composed of stacked cisternae that lack ribosomes and are somewhat curved (Fig. 18). The number of cisternae per dictyosome varies from 5 to 10, and intercisternal elements were observed. A polarity is apparent by the thickness of the cisternae: narrow on the molding or forming face and thicker on the final or maturing face (Fig. 17, 18).

The usual dictyosome cover or cap in interphase and early division is the nuclear envelope, the first of several features that emphasizes the intimate relationship of these organelles in this cell (Fig. 12-16). The progression of karyokinesis appears to include a sequence of nuclear-envelope evaginations correlated with a double appearance of Golgi bodies. In figures 12 and 13, early stages of envelope blebbing can be seen. Typical Golgi caps are seen to be shared by two dictyosomes (Fig. 15, 17, 18) at later stages of karyokinesis; in fact, if serial sections (Fig. 14-16) are studied, these cap vesicles are seen to be connected to the envelope by continuous membranes



Fig. 14. First of three serial sections through the nuclear region of S. unipunctata. Notice how the nuclear envelope (NE) projects outwards to form the cover vesicle (V) of the Golgi bodies (G).  
X 33,000.

Fig. 15. Second of three serial sections (see caption for Fig. 14). The vesicle (V) from the nucleus is still connected to the main body.. Golgi bodies (G) are found on either side of the vesicle.  
X 33,000.

Fig. 16. The nuclear vesicle (V) associated with Golgi bodies (G) appears to be detached from the nuclear envelope (NE) and shows the typical cover vesicle appearance between paired dictyosomes. X 33,000.

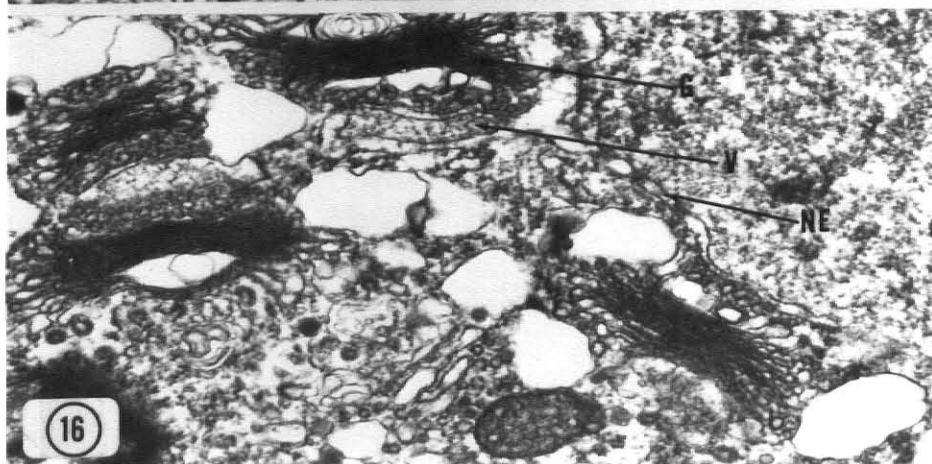
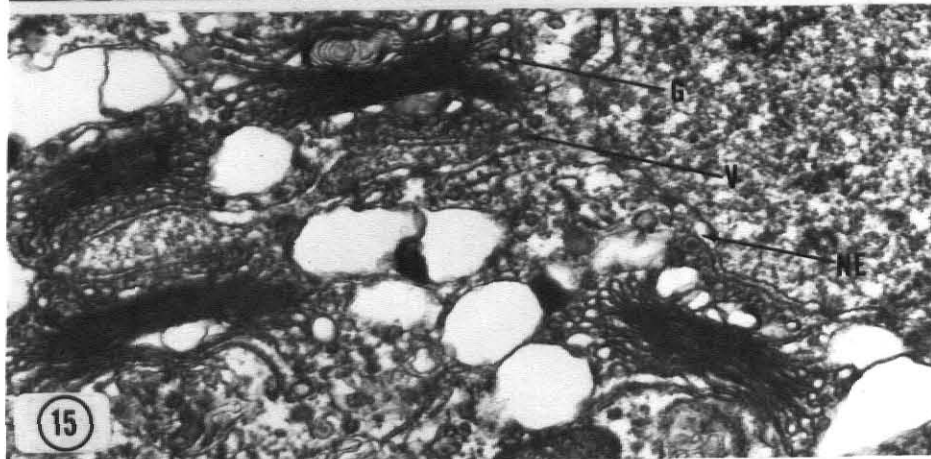
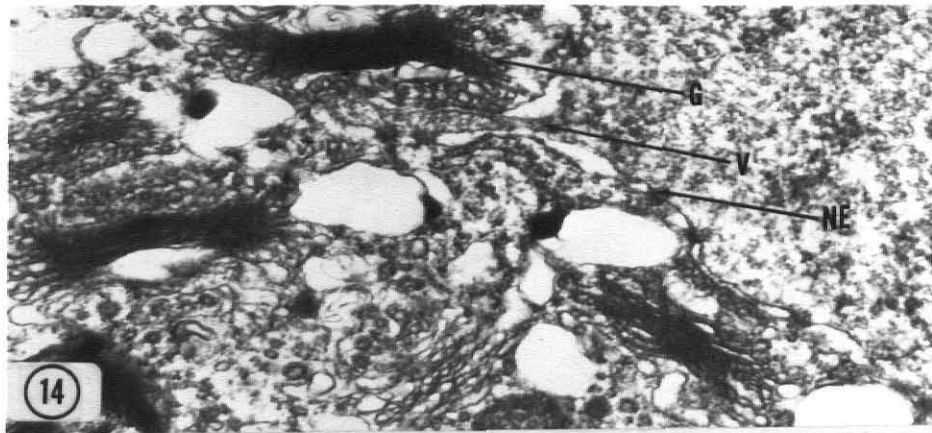
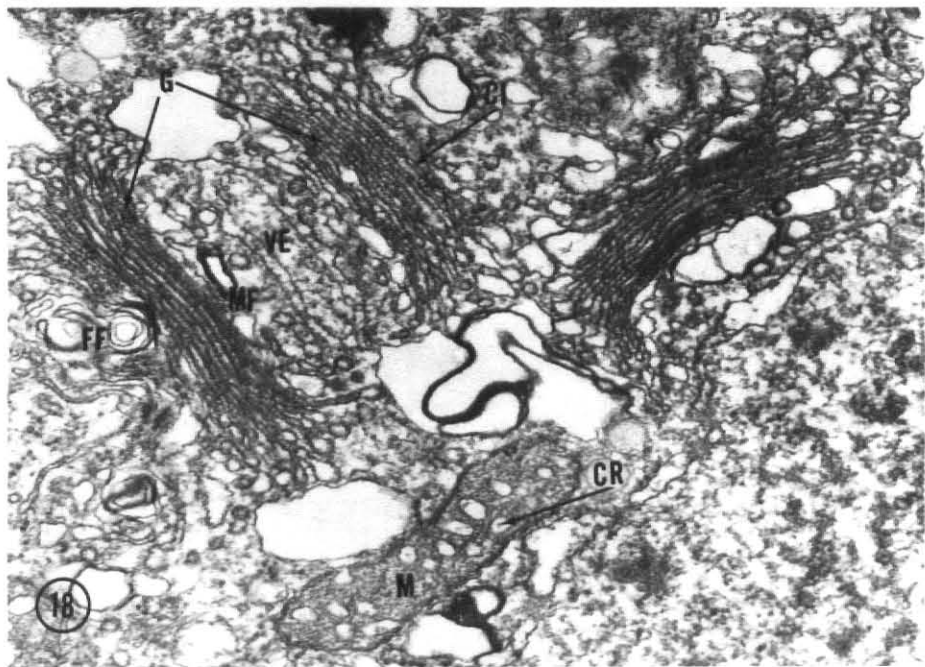
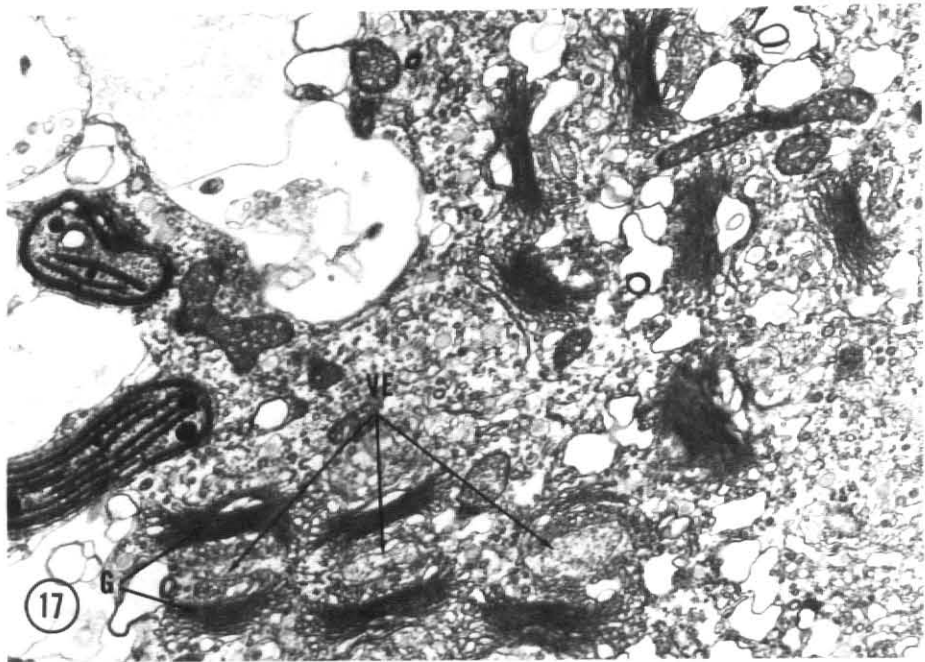




Fig. 17. Low magnification of paired Golgi bodies (G) separated by large cover vesicles (VE). Note the number of dictyosomes with associated vesicles and the tendency toward linear orientations (VE). X 19,000.

Fig. 18. High magnification of paired Golgi bodies separated by the cover vesicle (VE) bound by a double membrane. Individual cisternae can be seen to form the dictyosomes. Thin cisternae are located on the forming or molding face (MF) and thicker cisternae are on the maturing or final face (FF). A mitochondrion (M) with rounded cristae (CR) can also be seen. X 53,000.





and confluent nucleoplasm. By the end of division, most Golgi bodies have left their envelope-cap relationships, are free in the central cytoplasm, and are oriented toward a vesicle that is apparently of nuclear origin. These vesicles maintain a double-membrane form but no longer have either pores or annulae (Fig. 17, 18).

#### Mitochondria.

Mitochondria are randomly located in the cell in both central and peripheral cytoplasm. They can be found in the thin strands of cytoplasm surrounding the chloroplasts (Fig. 3) or near Golgi bodies (Fig. 17, 18). They are usually oblong in shape, often to lengths that are numerous times their diameter, and have rounded cristae (Fig. 3, 17, 18). They have a typical double membrane (Fig. 3, 17, 18).

## DISCUSSION

Chloroplast-microfilament relationships.

Early light microscopic observations by Mitrophanow in 1898 as reported by Fritsch (12) first described this organism as having two chromatophores arranged as half stars, each extending from the nuclear region toward each valve. Fritsch himself, however, suggests the possibility of numerous plastids. The question is clearly resolved by both movement and electron microscopy studies; several dozen individual plastids are present. That is, the changes of plastid positions are clearly movements of individual plastids, not changes in the shape of large, stellate chloroplasts.

Orientation, shape, and position changes are all known to occur in algae and higher plants, but have been comparatively little studied. Chloroplast movement in response to varying conditions of illumination has been noticed also in many Chlorophaceae. As a result of exposure to unidirectional light, the chloroplasts of Eremosphaera move toward the illuminated area. In Ulva, plastids move from the outer to the side walls upon exposure to darkness and back to the outer walls upon re-illumination (12). In other organisms, chloroplasts occupying relatively fixed positions react to similar situations by reorienting their chloroplasts according to the direction of the light or by changing in shape (see summary in 10). The chloroplasts of Maugeotia usually present their surfaces to the light and swing to a profile position when exposed to strong sunlight, a process that takes about 30 min. (12). The same effects are seen when Maugeotia is exposed to blue light, but when exposed to red light the opposite movement occurs. Changes in chloroplast shape have been observed in Acetabularia according to their endogenous circadian rhythm and photosynthetic activity (6). Further studies including

fine structure, action spectra, and phylogenetic comparisons are likely to be useful. Regulatory mechanisms should be elucidated since rates of photosynthesis could be greatly altered by such processes. Clearly Striatella is a valuable organism for such studies because it can apparently orient and move its plastids and shows some ability to modify plastid shape also.

Cytoplasmic streaming and other plastid movements have recently been associated with endoplasmic strands (1). These structures look like microfilament bundles and bind heavy meromyosin in arrowhead arrays, a finding that identifies them as containing actin (16). Further evidence for microfilament involvement in these processes comes from experiments demonstrating inhibition by cytochalasin B (5). The works of Schoenbohm (19) with colchicine and cytochalasin B strongly suggest that microfilaments are responsible for such movement. Microfilament-like bundles were found in association with chloroplasts in ultrastructural studies on S. unipunctata; only on one rare occasion was a chloroplast-related microtubule observed (Fig. 8). Studies, though of a preliminary nature, with cytochalasin B and colchicine give evidence for microfilament-related movement. Thus Striatella is the one of the few systems known in which a light-mediated organelle movement in a plant can be so well controlled in laboratory studies and thus appears to be a system worthy of additional study.

#### The mitotic apparatus.

Observations on cell division of S. unipunctata revealed extranuclear microtubules and a persistent nuclear envelope. The latter finding is contrary to the behavior of the nuclear envelope in some centric diatoms, also members of the class Bacillariophyceae where the nuclear envelope disintegrates during mitosis (14, 22).

The microtubules found in Striatella at the time of cell division were localized only in the cytoplasm. Bundles of microtubules were associated with nuclear clefts, and deformations seemed to be caused by microtubules which did not penetrate the nuclear envelope.

No chromosomal microtubules were observed. Dark condensations that were perhaps chromosomes were seen but did not show an orientation in any particular fashion. Thus it is only possible to suggest either the unlikely possibility of an amitotic division in Striatella or the possibility that, in spite of thirteen cells being chosen for division stages, sectioned individually, and observed in the electron microscope, metaphase and anaphase stages were missed. The question of what type of mitotic apparatus the pennate diatoms have remains unanswered.

#### Structure and formation of the Golgi apparatus.

Golgi bodies abound in diatoms, and Striatella is no exception. Their abundance can probably be correlated to their involvement in the formation of silica deposition vesicles and in the secretion of large amounts of mucilaginous material (7-9). The Golgi bodies in this cell are composed of typical flattened cisternae and peripheral vesicles. Each dictyosome is a stack of curved cisternae with apparent polarity: the forming or molding face with thin empty sacs and topped by a "cover" and the maturing or final face with thickened cisternae.

The Golgi bodies are found in the central cytoplasm, and their orientation changes according to the stage of cell division. At early stages of mitosis, single dictyosomes surround the nucleus and are closely opposed and parallel to the nuclear envelope whereas at late telophase or early interphase they pair and orient perpendicularly to the nuclear surface.

The study of the Striatella Golgi apparatus focuses primarily on the dictyosome cover or cap (see e.g., 13), a component of the organelle that usually receives little attention. Between each Golgi pair, a cover vesicle with a double membrane is found. Vesicles in this location have been previously described by other authors as portions of the endoplasmic reticulum and even as a fusion of the membranes of the Golgi apparatus and the smooth-surfaced endoplasmic reticulum (2). In some brown algae, the Golgi apparatus has been described as closely associated with the nuclear envelope (4); dictyosomes in the proximity of the nucleus are typical, but the location in Striatella is one of an actual apposition where the nuclear envelope takes the place of the usual cover seen in classical Golgi orientations.

The importance of this relationship is actually seen slightly later in the cell cycle. Here we have demonstrated by serial sections that the vesicle observed between paired dictyosomes is actually derived by blebbing of the nuclear envelope in such a way that nucleoplasm is contained within the final cover vesicle. Thus the forming region of the two dictyosomes is oriented toward covers that are actually opposite sides of the same flattened vesicle.

Since the nucleus-derived vesicle is situated on the forming faces of the paired dictyosomes, it is possible to suggest a role of nuclear components in the growth, development, and perhaps origin of Golgi cisternae. A similar functional involvement has been suggested by Flickinger (12) who studies enucleated giant amoebae and showed that Golgi bodies both disappeared during nucleation and were replenished following his resupplying of nuclei. Thus he suggested that the nucleus might be critical in Golgi formation by supplying RNA synthesized in the nucleus as an essential component to the

maintenance of the Golgi apparatus. The results obtained in Striatella support this dependency hypothesis, since the incorporation of nucleoplasm in the cover of the dictyosomes has been observed. Nevertheless, the role of the cover in Golgi function is understood very poorly, perhaps because of the likelihood that the cover functions in only a short part of total dictyosome metabolism.

Studies of the pairing phenomenon of Golgi bodies in diatoms is thus indicated as being one of particular import to furthering our knowledge of Golgi function. The classical observation of paired dictyosomes has been substantiated in other diatoms such as Pinnularia (8), so it appears that the diatoms in general are classical model systems with a Golgi-apparatus cycle that relates to its cover component particularly and integrates with the cell cycle. Although vesicles forming from out pocketings of the outer nuclear membrane have been associated with Golgi bodies (4, 9, 24) only the diatoms have shown the formation of vesicles with included nucleoplasm.

## CONCLUSIONS

1. The structure and cytology of S. unipunctata has been studied, and many essentially typical features have been established for organelles such as mitochondria, chloroplasts, microtubules, nuclear envelope, and nucleolus.
2. Chloroplast movement has been characterized as a karyostrophic controllable phenomenon that is microfilament dependent.
3. The stalk has been shown to be a secretion of highly fibrous nature originating by extrusion of material through the pores of the polar plate.
4. Golgi body pairing which is a characteristic feature of diatoms has been shown to relate to a double-walled vesicle that is derived by blebbing of the nuclear envelope in such a way that nucleoplasm is included. This vesicle functions like typical Golgi covers, and questions regarding the function of the cover are thus raised.

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## CHAPTER II.

Siliceous Structures and The Formation  
of Intercalary Bands.

## INTRODUCTION

The frustule of Striatella unipunctata is characterized by many intercalary bands in addition to the usual valves and girdle. Electron microscopy of such diatoms has not been done with either scanning or transmission microscopes, since interest has previously been focused on motile or centric diatoms.

The intercalary band becomes a particularly advantageous structure for the study of silica deposition, because they are being formed at almost all times of the cell cycle. Thus Striatella is a valuable organism for study of the very poorly understood process of diatom mineral deposition.

Moreover, the diatoms present one of the most beautiful cases of precise patterning. The current interest in positioning and patterning suggests that diatoms should certainly be studied in the light of questions pertaining to the methods both for positioning organelles and for generating patterns that are so critical in the cortical regions of protists.

The results of this study give information that shows precise membrane relationships to newly forming intercalary bands to the extent that a new way of approaching the question of pore determination can be presented.

## MATERIALS AND METHODS

Culturing methods and fixation schedules for transmission electron microscopy have been previously described in part I of this study.

Scanning electron microscopy of empty siliceous frustules.

Equal parts of "Dichrol" (a commercially prepared  $\text{H}_2\text{SO}_4$  and  $\text{Na}_2\text{Cr}_2\text{O}_7$  cleaning solution) were added to a suspension of diatoms and maintained for 60-120 minutes at  $55^\circ$ - $70^\circ$  C for cleaning of the siliceous frustule. This treatment digested away all non-siliceous material including the mucilagenous material that adhered the diatoms to the dish. So after shaking the dish, the diatoms and the growth medium were poured in tubes and centrifuged in a clinical centrifuge at low speed for 2-3 minutes. After decanting the growth medium, the organisms were rinsed three times with distilled water. A drop of the diatom suspension was then placed on glass-capped stubs that had been coated with a thin layer of adhesive that had been dissolved from a "Scotch" tape strip with acetone. The organisms on the stub were washed gently with distilled water and the excess water removed from the edge of the stub with filter paper after which they were coated with gold-palladium and observed under an Itek Scanning microscope, operated at 30 Kv. Photographs were made with Polaroid P/N55 4" x 5" sheet film.

Scanning electron microscopy of ion-etched cells.

Organisms were fixed in situ with 3% glutaraldehyde in synthetic sea water. They were then scraped from the dish, poured into centrifuge tubes, and centrifuged to make the total fixation time of 5 minutes. After decanting the fixative, 1%  $\text{OsO}_4$  in synthetic sea water was added for 15 minutes. After centrifugation and decanting, the cells were washed three times with distilled water. A drop of the suspended organisms was placed on a polished stub and coated with "Scotch" adhesive. They were then etched by directing a 15 Kv. -

beam of argon gas for 2-4 min at an 0.8 milliamp emission current at numerous parts of a stub so that some heavily etched regions were surrounded by more lightly etched areas. Etching was performed in a Itek scanning electron microscope which was also used at 30 Kv. for microscopic observations.

## RESULTS

Siliceous frustule.

The frustule of Striatella unipunctata, a pennate non-motile diatom, is rectangular with diagonally cut corners in girdle view and elliptical in valvar view (Fig. 1, 2, 3). The valve is broadly rounded (Fig. 2, VL) and contains a pseudoraphe (Fig. 2, PR) connecting two polar plates (Fig. 2, PP). Numerous intercalary bands run parallel to the valves and girdle (Fig. 1 to 3, IB).

The interrelationships of the intercalary bands is vital to the integrity of the organism since they comprise most of the surface. Two features are prominent for these precisely positioned and patterned structures. First, each is not a closed ellipse but rather is an elongated "U" or hairpin-shaped siliceous structure that interdigitates with its adjacent bands that are oppositely oriented; the arms of each band have tapering septa so that an internal view of the frustule shows an alternation of peripheral septum sizes (Fig. 2, 1); externally, one band shows a wide base that alternates with the end of the opposing band (Fig. 7). Second, the bands overlap each other in a shingled fashion. Each band in cross section appears as to be a T-shaped structure with a septum directed internally and a cross-member overlapping externally on one end and internally on the other (Fig. 8).

The girdle is seen in the central region of the cell by the time of cell division and, after karyokinesis, breaks away subsequent to formation of new valves (Fig. 2, 3, 10, GR). Devoid of both pores and septa, the girdle is still composed of overlapping bands (Fig. 11, 12, GL). An electron-dense substance (Fig. 11, 12, D) often appears on its external surfaces and is particularly thick at band junctions (Fig. 11, 12 GJ).

Striatella does not show a uniform porosity pattern throughout the



Fig. 1. Light micrograph of Striatella unipunctata. Very recently divided daughter cells, newly formed valves (VL) containing polar plates (PP), and adjacent new girdles (GR) can be seen in each cell, parallel to the intercalary bands (IB). X 850.

Fig. 2. Scanning electron micrograph of the siliceous frustule. This cell was probably at a late division stage; the upper part of the girdle (GR) has been lost. The valve (VL) can be seen as a lateral structure having a pseudoraphe (PR) and a polar plate (PP). The arrangement of the intercalary bands (IB) is shown from an exterior and an interior view. X 1,400.



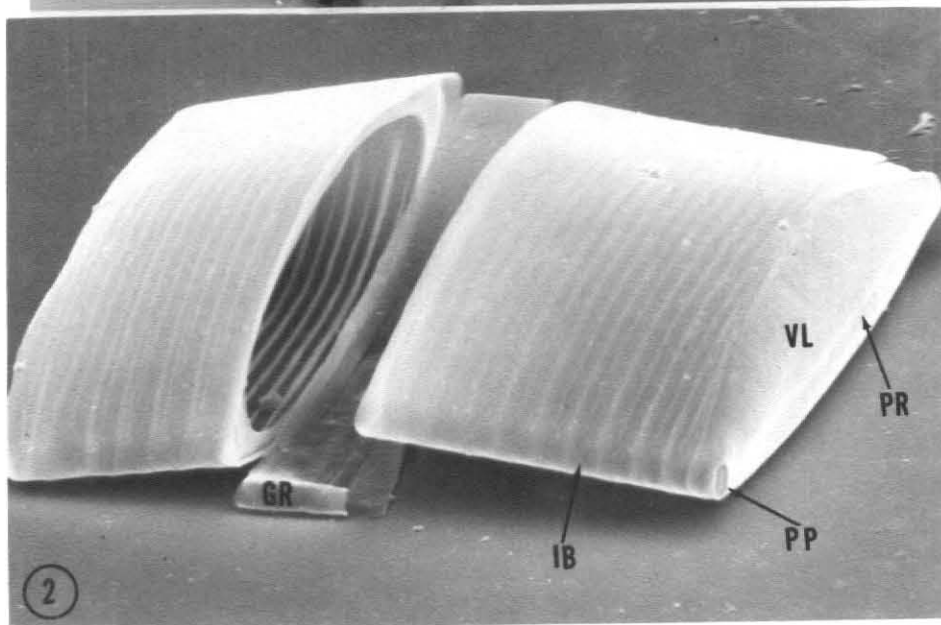
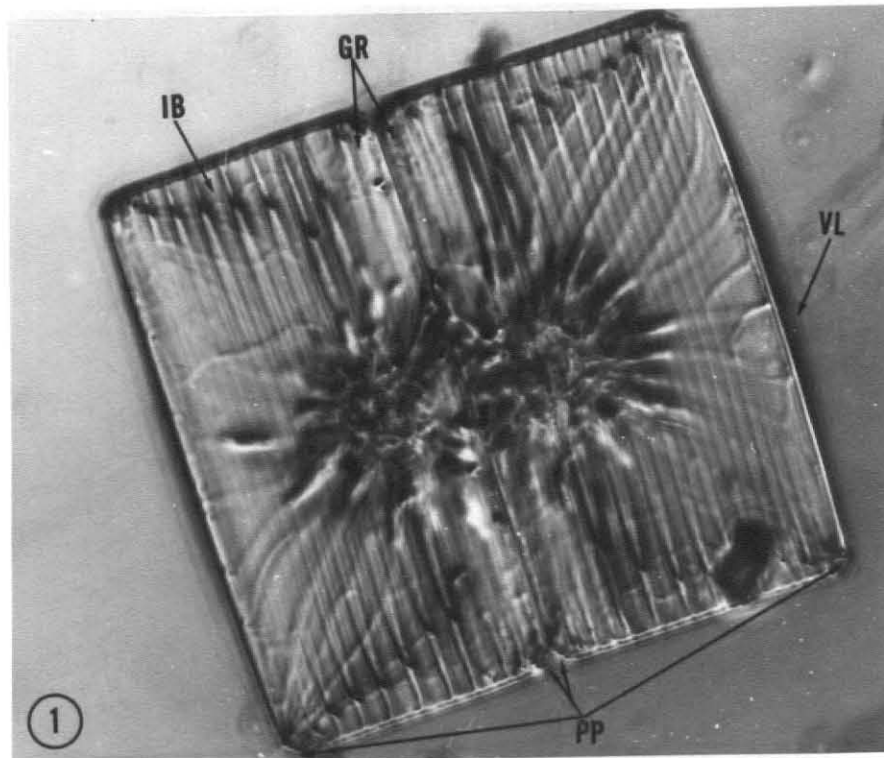
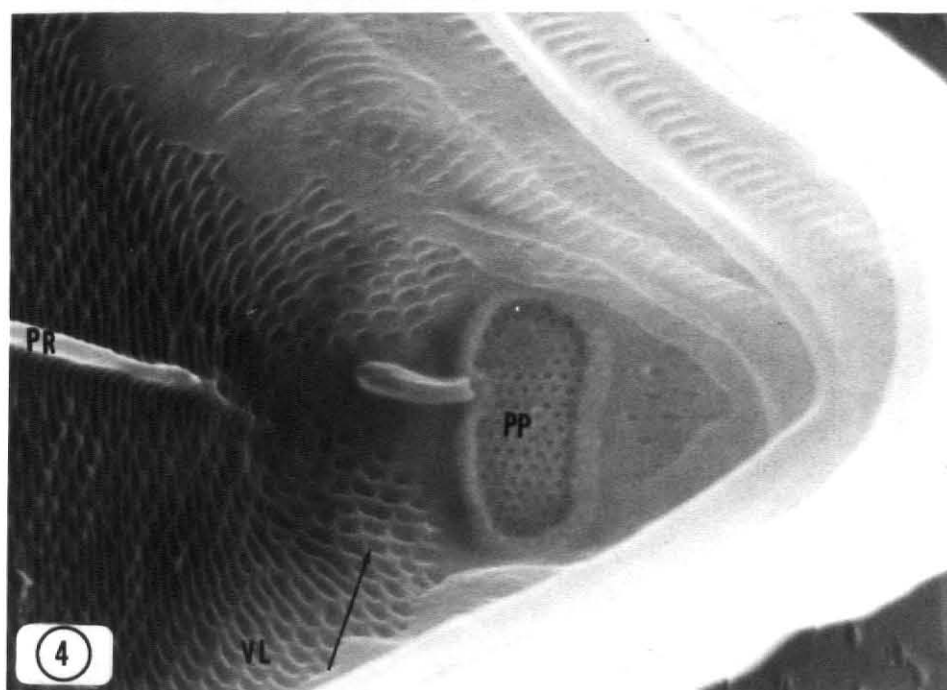
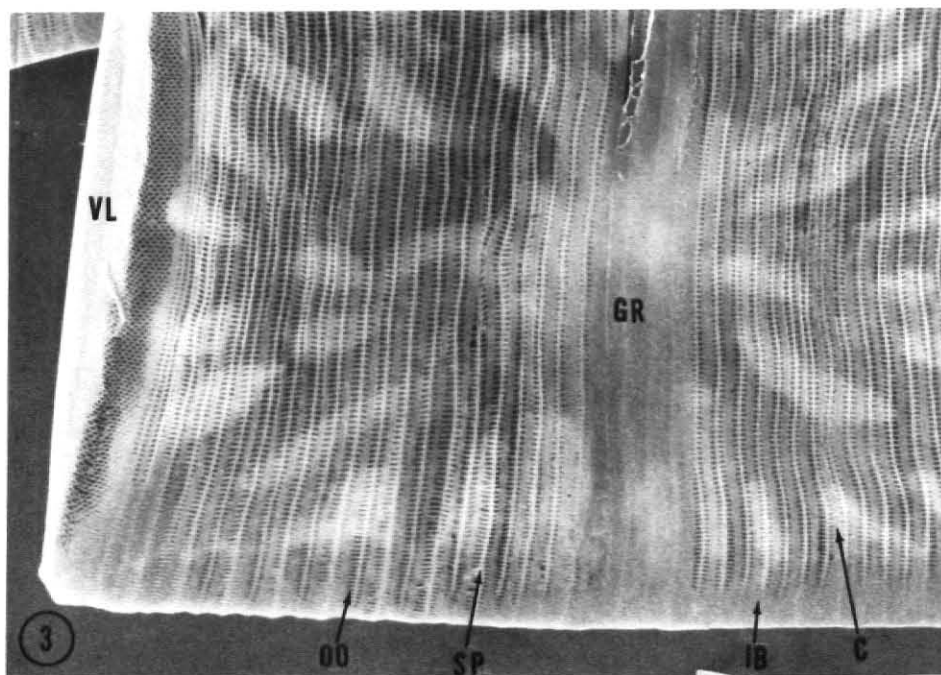




Fig. 3. Scanning electron micrograph of an argon-etched cell. This picture clearly shows the porosity pattern of the siliceous frustule of S. unipunctata. The porosity pattern on the valves (VL) is different from the one on the intercalary bands (IB) which show 2 rows of oval openings separated by a septum (SP) and bordered by a less porous band. The girdle (GR) is present as four bands that lack pores. Underneath the frustule some chloroplasts (C) can be seen. X 2,400.

Fig. 4. An internal view of a portion of a valve. The valves of S. unipunctata are divided longitudinally by a pseudoraphe (PR) which extends between the two polar plates (PP) located on both end of each valve (VL). A hexagonal pore pattern characterizes the valve (arrow). X 11,000.



entire frustule. Rather three patterns can be observed. First, each intercalary band has two rows of oval openings, one on each side of the solid septum; each opening is subdivided by frets that define small narrow pores (Fig. 3-6). Second, the valves have a generally open porosity that is hexagonally ordered (Fig. 3-4). Third the polar plate has a porosity showing a low percentage of open area (compared to the valve) and a hexagonal ordering (Fig. 4, PP).

The plasma membrane contacts only the septa of the mature intercalary bands. In certain regions numerous villus-like projections are clustered (Fig. 9).

#### Cellular structure related to silica deposition.

The plasma membrane of Striatella makes very close contact with the siliceous frustule at the girdle region where cytoplasm extends to reach the newly forming intercalary bands.

In a section through the center of the valvar plane, a particular cellular structure directly involved in silica deposition can be seen. T-shaped vesicles (Fig. 10 to 12, TVE) contain newly formed septa or septum precursors. Towards the right of the vesicles, septa at later stages are found. Each vesicle contributes only one U-shaped band. These T-shaped vesicles show certain structural relationships that are probably quite significant to their apparent roles of determining the environment, the position, and the porosity of early intercalary band structure. First, the earliest form seen is, in cross-sectional view, a "head" with a thin, tapering (septum) shaft; whether this is siliceous is not known. Second, the "head" is in close apposition to the vesicle membrane which is in contact with the plasma membrane. Third, one edge of the vesicle-plasma membrane complex contacts the interior surface of the slightly older band in the same way that the overlap of the two bands



Fig. 5 & 6. Detailed views of the pores and frets of the oval openings. Sections through intercalary bands show the pores (PO) and frets (FT) in oval openings (OO) which are located on either side of the septum (SP). Transmission electron micrographs. X 19,000 and 23,000 respectively.

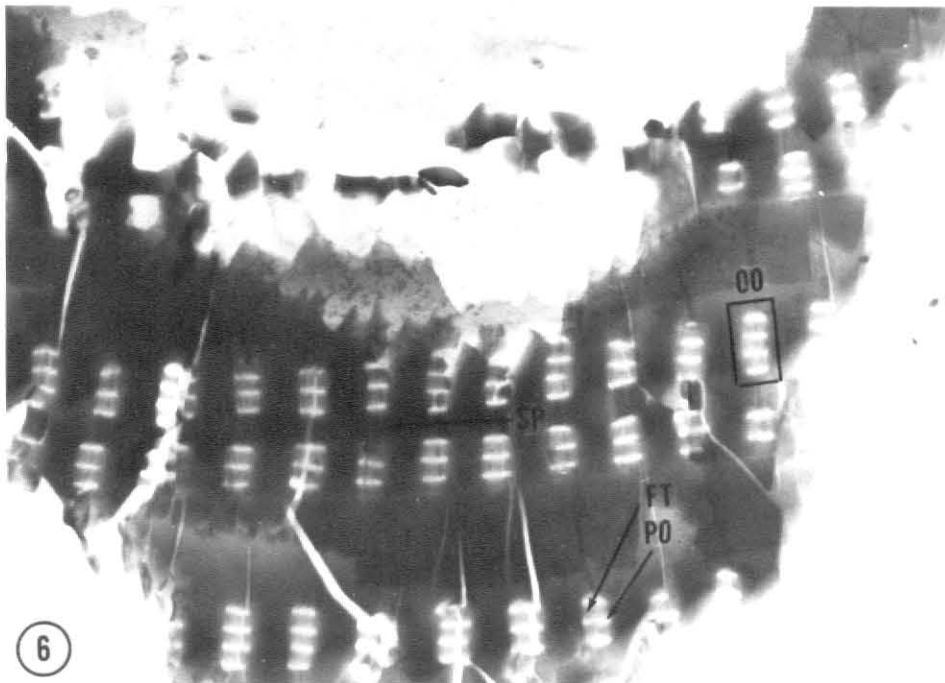
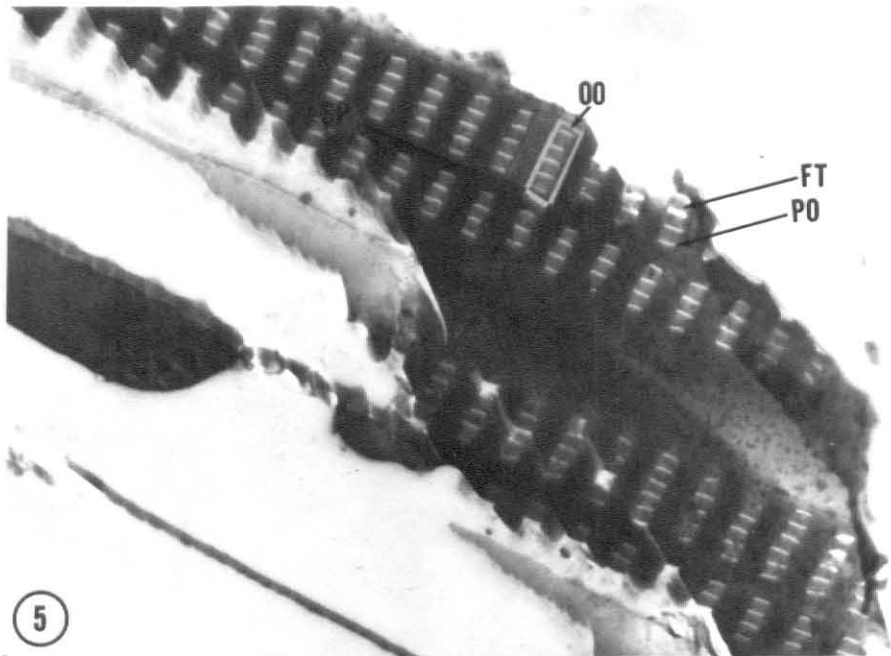
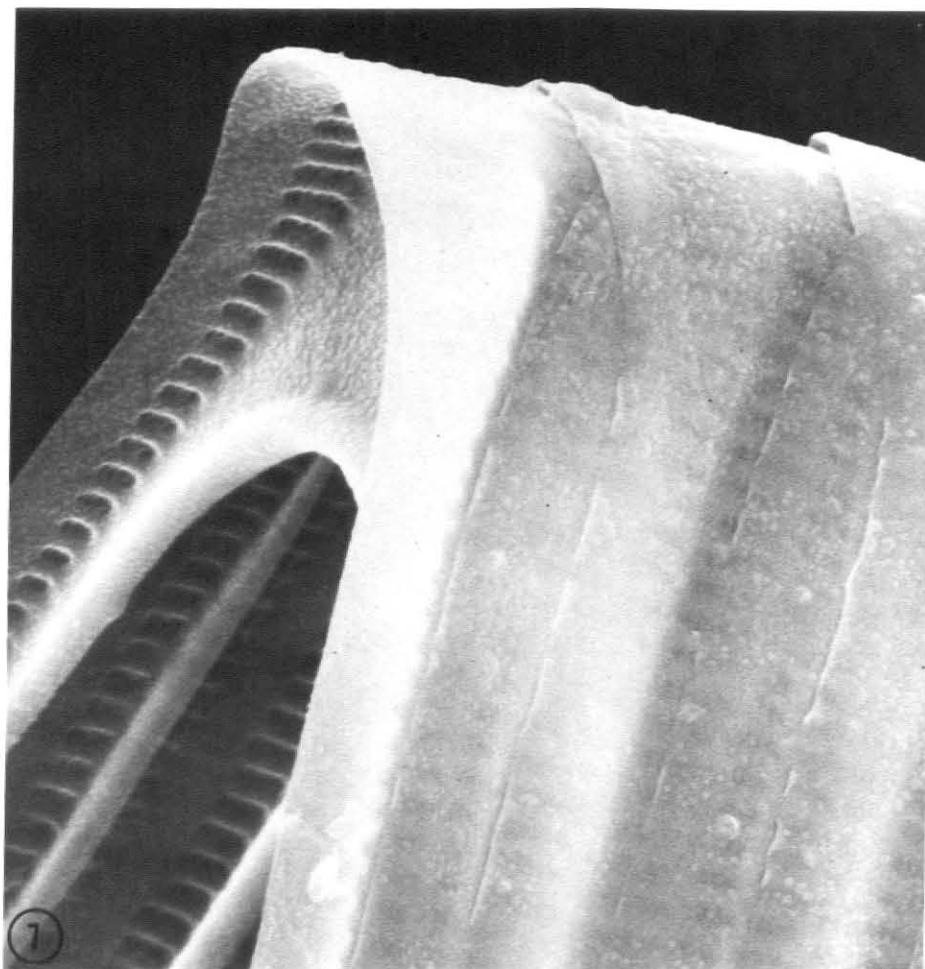






Fig. 7. Scanning electron micrograph of the periphery of a frustule.

Note the alternation of wide and narrow ends of adjacent intercalary bands seen from the exterior and the septa and pores seen interiorly. X 19,000.



will finally be. Fourth, the area of vesicle-plasma membrane between the "head" and older band contact is about the same dimension as the pore length in mature intercalary bands.

Adjacent older bands (Fig. 11) still do not show full development of intercalary-band size of either portion of the T-shaped cross section. Nevertheless, the band is now totally extracellular, the vesicle having apparently ruptured.

Attempts to view earlier stages of vesicle formation have been unfruitful. However, structureless vesicles are typically present near where a new one will be needed but contain no formed structures (Fig. 11, 12, VE).

The T-shaped vesicles are not only found in the central region of the cell; figure 10 shows a T-shaped vesicle at the corner of a daughter cell recently separated from the girdle. Figure 1 is a light micrograph of a cell at a similar stage when division has just been completed. These findings suggest an almost continuous presence of such vesicles throughout the entire cell cycle, a phenomenon quite compatible with expected growth in this species.

#### Pore size and number.

The oval openings on each side of the septum contain small frets and pores which vary in size and number. Usually there are fewer pores on the side of the septum closer to the girdle; this side would be the one on the side of the T-shaped vesicle that is in contact with the older intercalary band. The length of individual pores varies, the longest usually being in the center of the openings. The pore length averages five times the pore width; the fret width averages three times the pore width (Table I).



Fig. 8. Cross section of three intercalary bands. Intercalary bands show pores (PO) and their separating (FT) between their overlapping junctions (IJ) and their septa. X 12,000.

Fig. 9. Detail view of soft structures extending to the septa (SP) and up to the intercalary band junctions. Note the specialized intertwining projections. X 23,000.

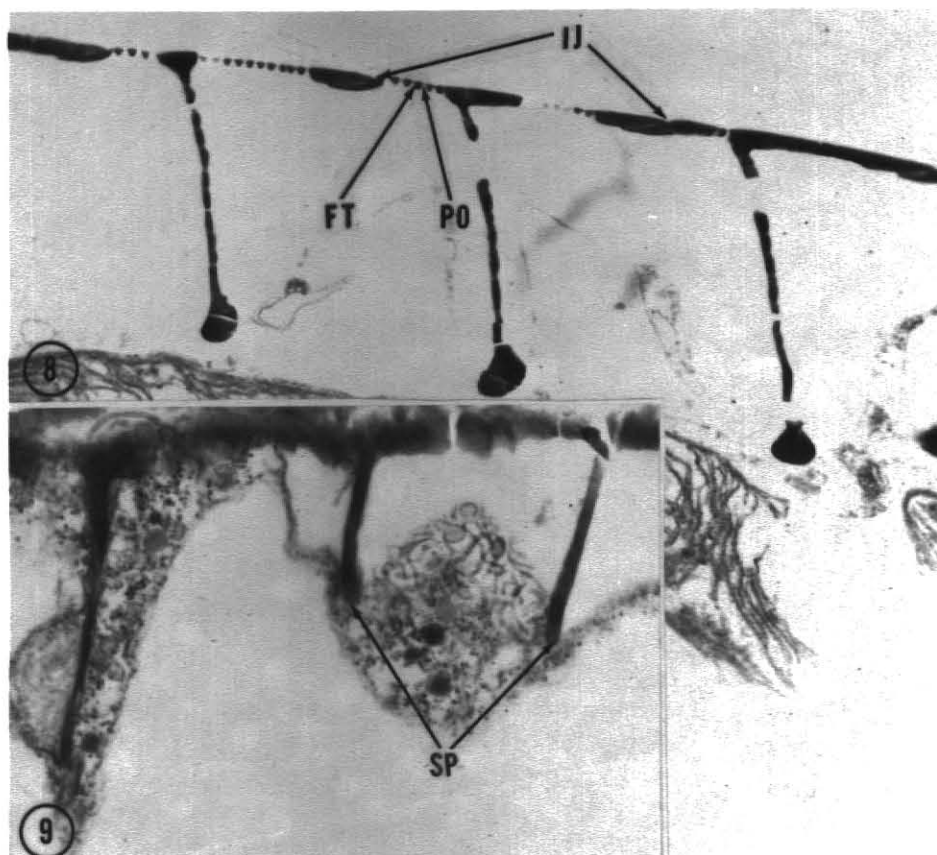






Fig. 10. Survey section through a cell nearing division. By the time of cell division, the girdle is visible in the central region of the cell. The cytoplasm (CT) of the organism now extends up to the siliceous frustule at the girdle (GR). Precursor vesicles for the silica deposition, the "T" vesicle (TVE) are seen on both sides of the girdle. Compare the septum (SP) of a mature intercalary band with the precursor. Chloroplasts (C) are clustered around the central cytoplasm. X 12,000.

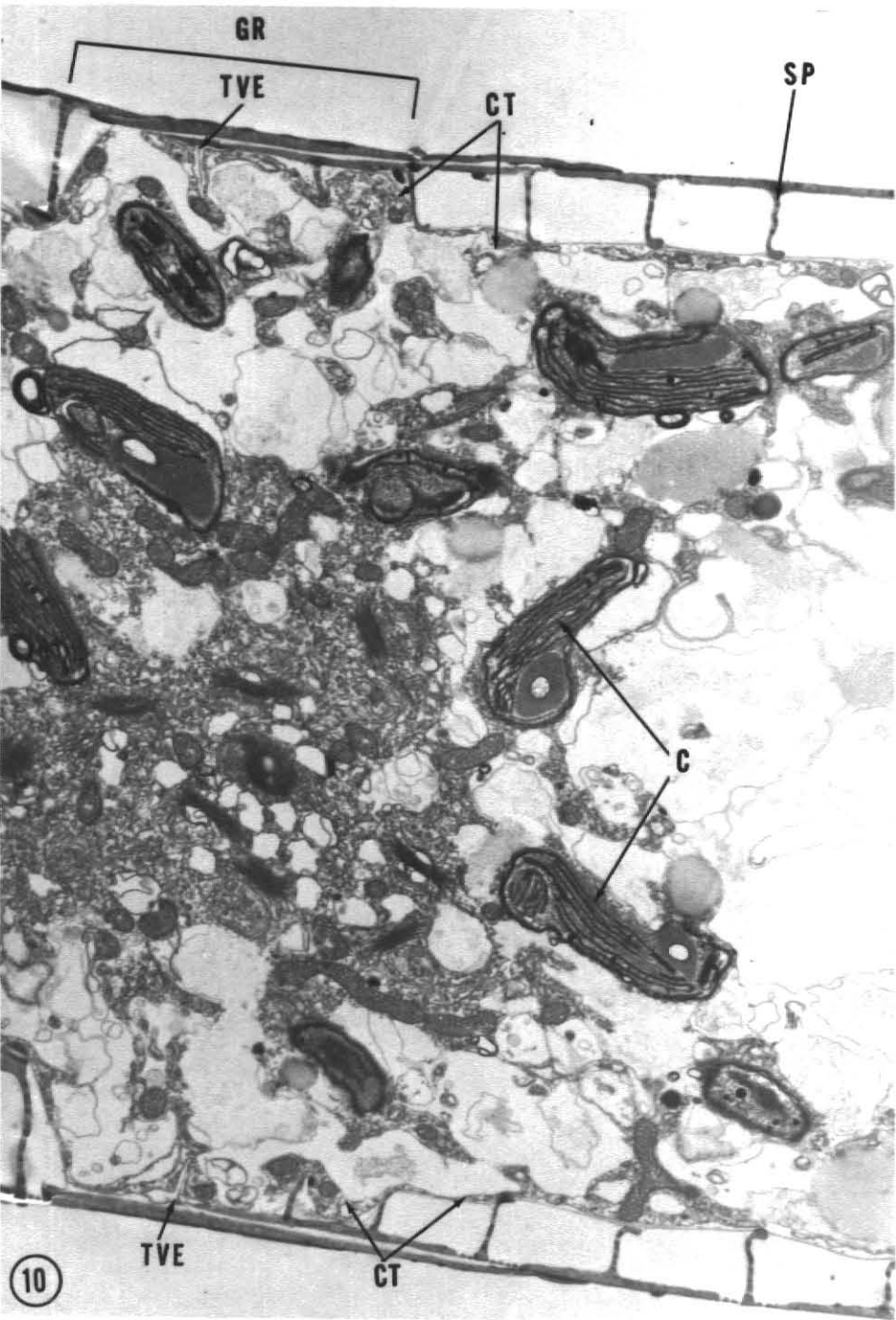




Fig. 11. Enlarged view of a section serial to that of Fig. 10, the upper girdle region. Two girdle junctions (GJ) are seen and delineate the girdle bands; electron-dense material (D) is typically deposited on the girdle. The T-shaped vesicle (TVE) is typically positioned to contact the adjacent band and contains a typical band precursor structure. The septum (SP) is a part of an immature band that is extracellular and has perhaps a small amount of cellular debris at its junction (IJ) with a mature band. A vesicle (VE) is seen in the location where a new T-shaped vesicle should appear.

X 24,000.

Fig. 12. Enlarged view of lower girdle region of Fig. 10. A T-shaped vesicle (TVE) is similarly positioned and contains a precursor again in apposition with the vesicle-membrane and the plasma membrane which are closely positioned. A junction of girdle bands (GJ) and dense material on the exterior girdle surface are visible.

X 39,000.

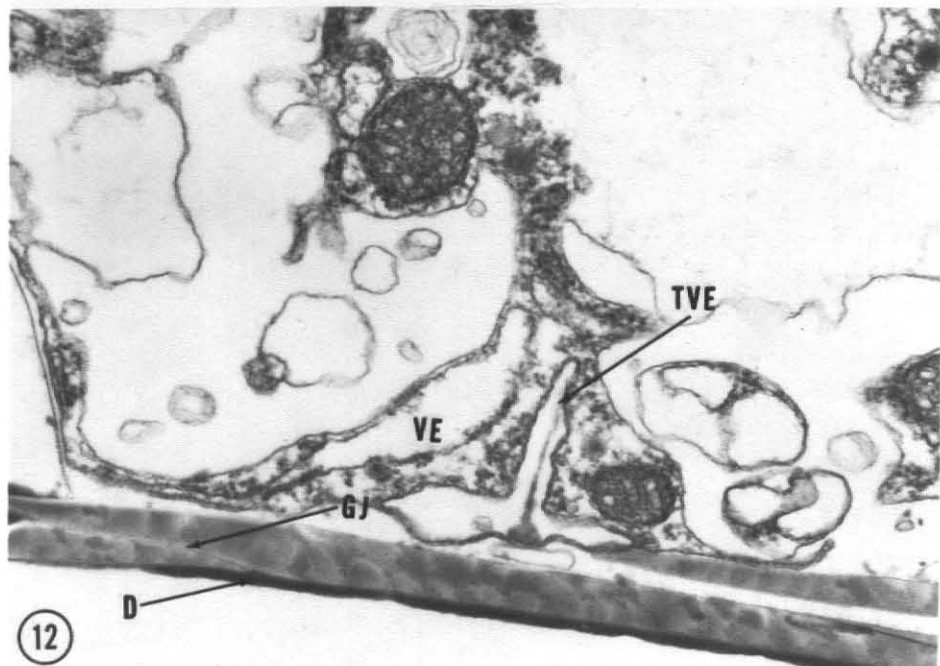
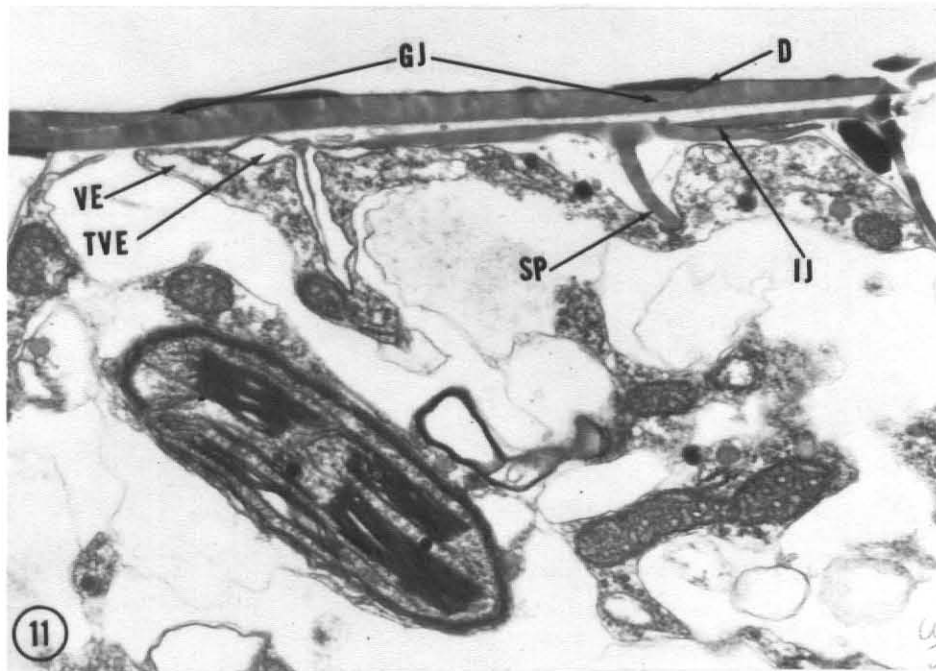




Fig. 13. Section through a recently separated daughter cell. A small T-shaped vesicle (TVE) close to the valvar region (VL) of the cell, and a mature septum (SP) are seen in this section.  
X 36,000.

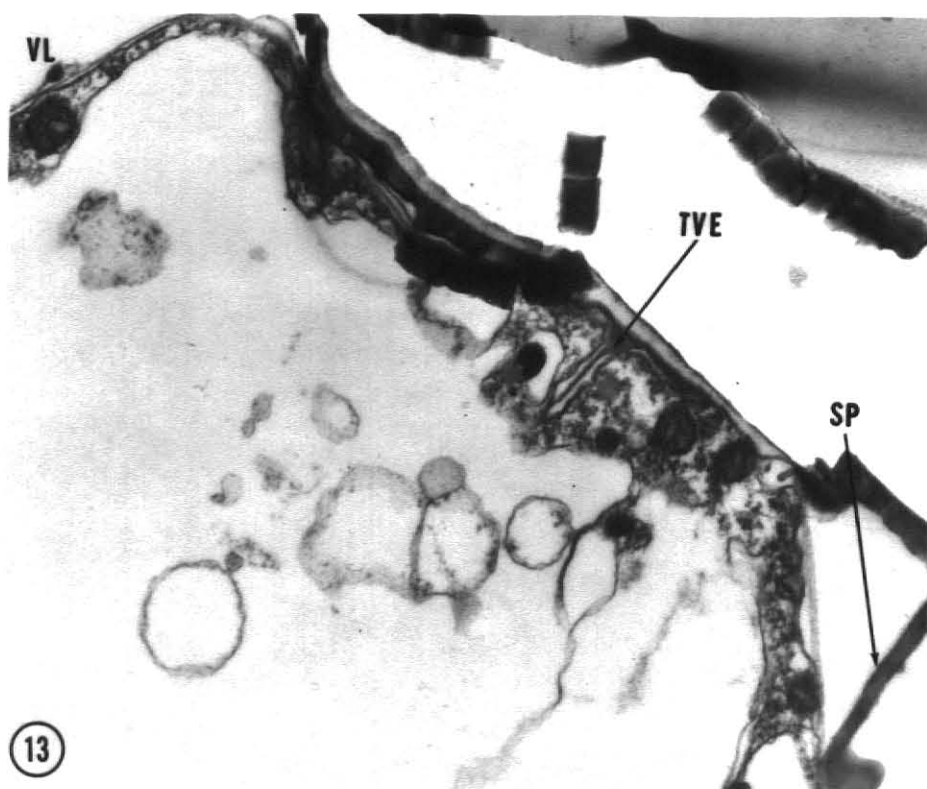




Table I. Pore Dimensions in Intercalary Bands in *Striatella unipunctata*. Each clone-organism line indicates a separate transmission electron micrograph was used. Note that three clones, four organisms, and six micrographs were used in obtaining these data.

ORGANISM NO.	PATTERN	PORE WIDTH			PRT WIDTH			PORE LENGTH		
		RANGE	AVERAGE	# MEASURED	RANGE	AVERAGE	# MEASURED	RANGE	AVERAGE	# MEASURED
Clone 1, organism 1	3 to 7	7.6-19 nm	13.13	96	28.5-47.5 nm	37.40	84	57-86.07 nm	68.70	94
Clone 1, organism 2	4 to 6	7.6-19 nm	12.64	43	32.3-38.0 nm	36.10	29	42.75-71.25 nm	58.50	47
Clone 2, organism 1	3 to 4	5.7-15.2 nm	9.50	23	28.5-45.6 nm	36.80	17	42.75-71.25 nm	58.80	21
Clone 2, organism 2	3 to 6	5.7-15.2 nm	8.19	107	32.3-47.5 nm	37.40	79	14.25-63.84 nm	49.40	106
Clone 3, organism 1	3 to 5	5.7-13.3 nm	9.85	11	28.5-38.0 nm	33.40	18	42.75-63.84 nm	57.90	19
Clone 3, organism 2	3 to 4	9.5-20.9 nm	14.38	30	28.5-45.6 nm	35.30	23	14.25-71.25 nm	58.00	32
OVERALL AVERAGE		6.9-16.783 nm	11.70		29.76-43.7 nm	36.10		35.625-71.25 nm	58.5	

## DISCUSSION

Striatella unipunctata is a valuable organism for the study of silica deposition phenomena. In contrast to other diatoms where silica deposition takes place largely in a short part of the cell cycle when valves are being formed, this organism is forming intercalary bands throughout most of its cell cycle. Thus, almost any cell studied shows not only a newly forming band but also an immature band, both juxtapositioned to fully formed mature bands.

Furthermore, the intercalary band is a highly structured entity. It not only has an elaborate shape but also a precise porosity pattern. This particular structure therefore allows close observations of both position-determining and pattern-determining phenomena at the high level of precision that diatoms possess. Phenomena of positioning and patterning have remained poorly understood, and, especially in studies of protistan cells, present a strong and current emphasis of research effort.

### The formation of intercalary bands.

Formation of new bands is a process that involves a sequence of events. Although the following list may not have the complete or proper sequence, certainly each step indicated must be included.

1. The vesicle forms. The indication from our study is that the vesicle is first seen in the proximity of its needed location without a particular shape and without any internal content identifiable.
2. The vesicle is positioned and shaped. In addition to its "T" shape, the vesicle must take up a position appropriate to the adjacent intercalary band and in close apposition to the plasma membrane.

3. A band precursor is formed. The earliest indication of an intercalary band is composed largely of a septum and a small "head."

4. Band porosity is determined.

5. The T-shaped vesicle ruptures. With this event, the immature intercalary band becomes extracellular.

6. The band is fully silicified. Not only is the structure enlarged, but it is further structured at the same time that its positional relationship to the previously formed band is maintained.

Determination of the pattern and positioning of new intercalary bands.

The above process is clearly separated into a period that takes place inside a vesicle and a second stage that takes place extracellularly. Drum and Pankratz (3) first described the presence of a "silica deposition vesicle" in their study of Gomphonema parvulum. The studies of Striatella substantiate their claim for an intra-vesicular stage, but enlarge upon that concept by showing that silica deposition is completed extracellularly and that the earlier stages take place not only in a vesicle but in intimate contact with the vesicle membrane at the particular areas where pattern determination is particularly required. Although the former indication of extracellular deposition is not surprising, the later observation is a critical one, because it now allows an approach to a consideration of how diatom patterns are actually formed.

The indication is that the pattern of silica deposition in which porosity size is determined is probably best understood as a function of the T-shaped vesicle membrane and/or the plasma membrane. Not only are these membranes intimately related to the forming band, but the vesicle contact with the partially formed band is also established. In addition a membrane area is defined between that point of contact and the band precursor and is

similar to the dimensions of the ciliate rosette. The data for intercalary band pore size have been determined by a large number of measurements and, not only established the pore size, but show that the average pore width is 11.7  $\mu\text{m}$ , a dimension quite similar to the rosette spacing where particles of 12  $\mu\text{m}$  are separated by a similar sized spaces. Thus, it can be hypothesized that intra-membrane elements define rectangular patterns of the approximate size of the pores. Instead of the circular pattern that the rosettes contain, this pattern would be a rectangular one that has 6 to 12 intra-membrane elements bound together by linkage molecules to make a rectangle of rather uniform width. The observed variation of pore length can be attributed to a variability in the number of such elements included in the unit structure and to some perturbation of its rectangular shape by local forces present in the membrane at the time of pattern determination.

Further studies are thus indicated in the precise area of the T-shaped vesicle where pores are to be located. If membrane specialization of the type shown by Bardele (1) can be demonstrated by transmission electron microscopy, then precise freeze-fracture microscopy would be warranted to show membrane particle organization.

The band precursor should also be studied further. Is it composed of siliceous or organic material? Drum (2) seems to favor a non-siliceous early component being found and bases his contention on the presence of material not digestible by hydrofluoric acid in Golgi vesicles that are likely to become silica deposition vesicles. Other information in regard to diatoms is lacking. However it is generally conceived in mineralization processes that organic matrices are formed first. For example, studies of the scale and base plates found in algal cells, particularly in the formation of coccoliths, show good examples of matrices that may be cellulosic in nature (5, 8).

Eastoe (4) reviews the subject of calcification and suggests that the organic-matrix concept is characteristic in such systems. Certainly questions of whether the siliceous structure of diatoms is first determined by an organic precursor or whether the frustule is entirely siliceous are worthy of study in the future.

Other questions remain, however. The mechanism of vesicle orientation to the previously formed band is unknown, as is also the reason for the placement of the band precursor in its particular location within the T-shaped vesicle. These questions and the precision with which they can be viewed again show the merit of Striatella as an organism that can profitably be studied further in order to approach questions regarding the mechanism of positioning and patterning that remain enigmatic in cell biology.

## CONCLUSIONS

1. New intercalary bands are first formed inside a T-shaped vesicle whose location appears to be precisely determined and whose membrane is apposed to the area where pore patterns will be specified.
2. Membrane components are suggested to be the determinants of silica patterns.
3. Striatella is a valuable organism for further studies of mineral deposition and of pattern-determining mechanisms.

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Structure-function Relationships  
in the Marine Diatom, Striatella unipunctata

by

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AN ABSTRACT

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## ABSTRACT

Transmission and scanning electron microscopy were used for studies on fine structure of Striatella unipunctata. This marine non-motile pennate diatom was cultured and cloned in the lab in Instant Ocean synthetic sea water.

Typical features were observed for mitochondria, microtubules, chloroplasts, nucleolus, and nuclear envelope. Microfilament bundles associated with the chloroplasts are thought to be the motive force involved in the karyostrophic chloroplast movement characteristic of this species. The fine structure of the polar plates consists of numerous pores; the function was shown to be the secretion of a mucilagenous stalk or holdfast. The pairing of Golgi bodies was associated with a double-membraned vesicle derived from the nucleus and containing nucleoplasm. This vesicle forms the Golgi cover which function is still unclear.

Studies on the siliceous frustule of Striatella revealed the presence of a T-shaped vesicle found immediately underneath the frustule in places where new intercalary bands of silica deposition were being formed.

Striatella has been shown to be a particularly valuable organism for two areas of study. First, function of the Golgi apparatus can be investigated, particularly in regard to duplication of dictyosomes and to function of the Golgi cover component. Second, silica and mineral deposition in precisely specified patterns can be studied, particularly during the formation of new intercalary bands.